Chapter 5

Seed Testing

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Introduction

Seed testing is the cornerstone of all other seed technologies. It is the means by which we measure the viability and all the physical factors that regulate the use and maintenance of seeds. Everything that is done with seeds should have some test information to guide the work and ensure high quality. Seed tests tell if a crop of seeds is worth collecting, if handling procedures are correct, and how many potential seedlings are available for regeneration.

The earliest form of seed analysis, the cut test, is still often used today. Before seeds are collected in the field, some seeds are cut open with a knife or razor blade to see if their internal tissues are fully developed and undamaged. This analysis is made more accurate in some cases by the use of a hand lens. It is also used for simple analysis during extraction and cleaning, or after germination to determine if the ungerminated seeds have deteriorated or remained dormant. Although the cut test is often very good at producing some information quickly, it is limited in the amount of information it can supply and it lacks accuracy compared to more sophisticated procedures. Therefore, it should never be taken as a substitute for a formal laboratory analysis.

Sampling

Formal seed analysis begins with the sampling of the seedlot. The Rules for Testing Seeds (AOSA 1996) and the International Seed Testing Rules (ISTA 1996) both give instructions on how to draw samples from a seedlot so that the sample is representative of the entire seedlot. Representative means that any tests conducted on this sample will accurately estimate the mean value of the lot quality.

Sampling can be done with the hand or with a seed probe, also known as a trier (figure 1). If a probe is used, it must be long enough to reach to the farthest edge of the container. A probe has gates that prevent seeds from entering until the probe is inserted the full dimension of the container. The probe should be inserted into the seed container with these gates closed. Otherwise, seeds from the upper layers will fill the probe as it is inserted and the bottom layers will not be sampled. Once the tip reaches the bottom or far side of the container, the gates should be opened and the probe gently turned back and forth to help the seeds fall in. Then the gates should be closed gently, not forced, so that any seeds that are caught in the opening and are preventing the gates from closing fully (figure 2) are not crushed. (Mechanically damaged seeds would bias the sample.) After the probe has been withdrawn from the seed container, it should be held horizontally, with the gates facing upward. Then the gates should be opened gently and the probe shaken gently back and forth, so that seeds caught in the gates will slip down into the probe and the gates can be safely closed. Finally, the probe should be emptied by pouring the seeds out the top of the probe and into a second container (figure 3). This sample is the first primary sample.

If there is only 1 container, primary samples should be taken until there are 5 primary samples. When more than 1 container holds the seedlot, at least some of the other containers must be sampled. When there are between 1 and 5 containers, all containers should be sampled, at least 1 probe from each container. When there are more than 5 containers, 5 of the containers plus 10% of the remaining ones should be sampled. It is never necessary to sample more than 30

containers. (It would be rare that a forest seedlot would need 30 containers or more, or possibly even 20.) All of the primary samples are then placed together to make up the composite sample.

Sampling by hand is sometimes necessary when the seeds will not flow into the probe because of their size, shape, or surface texture. Sampling by hand can be done by inserting the open hand (figure 4) into the seeds, closing it once the point of sampling is reached, and then withdrawing it closed. The seeds are then placed in a second container to form the composite sample, just as in sampling with the probe. At least 5 handfuls must be taken, and all levels must be sampled. When the hand cannot be inserted into the seedlot, the seeds can be poured from one container into a second, stopping at a minimum of 5 evenly spaced intervals and removing a handful of the seeds for the composite sample.

The composite sample, whether taken with a probe or by hand, is usually too large to submit to a seed laboratory for analysis. The composite sample is, therefore, mixed and divided to obtain a submitted sample. This procedure is very important and must be done correctly for the results to be accurate.

The composite sample can be mixed either mechanically or by hand with rulers. Hand-mixing the composite sample is done by pouring the seeds into a cone on a flat, clean surface. An open file folder makes a good work surface that can be picked up to return the seeds to a container. With one ruler held stationary against the seeds, the second ruler is used to pull the outer edge of the pile up to the top of the pile, allowing the seeds to roll down the sides and over the top of the stationary ruler (figure 5). The full pile is thoroughly turned over and all layers mixed together. This procedure should be repeated for 1 full minute. Then the pile should be divided by cutting the cone in half and then into quarters. The quarter is then weighed to see if it is enough for the sample. If not, then another quarter, an eighth, or a smaller fraction is taken until the minimum weight is obtained (figure 6).

Hand mixing can be replaced by mixing with either a soil divider or a gamet divider (figure 7). These devices can save a substantial amount of time and also, by reducing the tedious nature of the work, increase the likelihood of doing a quality job. The seedlot needs to be poured through the divider 3 times. When the gamet divider is used, the motor must not be turned on until the seeds have been poured completely into the hopper. Once the seeds are cleared out of the machine, the motor must be turned off before the seeds are poured back into the hopper for the next pass. The seedlot is then divided in half, then quarters, eighths, and so forth, to obtain the correct weight for the submitted sample, just as in the hand mixing and dividing.

The size of the submitted sample for some species is stated in the Rules for Testing Seeds (AOSA 1996) and is twice as large as the minimum amount for the purity test. This amount is different for each species and the rules need to be consulted to be sure the correct amount is submitted for purity tests that are to be done according to the rules. A smaller sample of seeds can be submitted, but the test will not be according to the rules and the accuracy cannot be assured to the same degree as a test that is done according to the rules. If a species is not listed in the rules, an amount that contains 2,500 seeds should be taken. This amount can be estimated by counting out 100 seeds and multiplying their weight by 25. Under the AOSA rules, samples can be as small as 600 seeds when only germination is tested. It is important to work quickly when drawing the sample, if the submitted sample is to be tested for moisture content. This will prevent the gain or loss of moisture from the air. Once obtained, the submitted sample should be

put in a moisture-proof container to maintain its true moisture content until it is sampled and tested at the laboratory. Plastic bottles with tight-fitting lids or tightly closed plastic bags of at least 0.1 mm (4 mil) thickness are adequate. Metal containers can be used but are harder to find. Glass containers should not be used; they easily break in transport, allowing the samples to be exposed to the air or, worse, mixed together.

Sample Identification

Assignment of a test number is the first step in handling every seedlot that is received in the laboratory. This number allows for the orderly tracking of the test sample among the other samples in the laboratory. A typical test number indicates the test year and an accession number. For example, the 300th test conducted in 1996 would have a number such as 6-300.

Moisture Tests

Moisture tests must be the first tests conducted on samples when they arrive at the seed laboratory. Once a sample container is opened and work begun, the seeds will likely gain or lose moisture in exchange with the ambient air. The standardized laboratory test for moisture content is the oven method (ISTA 1996). This procedure was determined, after many years of research, to be a best estimate of moisture for general testing work (Bonner 1972, 1981, 1984, 1992; Buszewicz 1962; Hart and Golumbic 1966). This test is made on 2 subsamples containing 3 to 5 g of whole seeds. These 2 samples are placed in containers with lids and weighed to determine the wet weight (figure 8). Then they are placed in a forced-draft drying oven (figure 9) for 16 to 18 hours at 105 ± 2 °C. The lids are removed during drying but are also placed in the oven. The samples are then placed in a desiccator to cool for about 20 minutes before being weighed a second time to determine their dry weight. The lids are placed on the cans while cooling and weighing. The loss of weight represents the weight of water in the undried sample. This water weight is divided by the wet weight to obtain the percentage moisture content on a wet-weight basis. The percentage moisture is expressed on a wet-weight basis because this value most accurately represents how much of the seedlot is water. Therefore, the buyer knows how many seeds are purchased and how much water is purchased. For example, when the price per weight is the same, a pound of seed at 7% moisture content is a better value than a pound of seed at 9% moisture content. For example, a 100-kg seedlot (or a 100-lb seedlot) at 7% moisture contains 93 kg of seeds, whereas the lot at 9% moisture contains 91 kg of totally dry seeds, 2 kg less.

Some larger seeds and seeds with impermeable seedcoats need to be cut to make an accurate test (Bonner 1974, 1981, 1992). If the seed is not cut open, the moisture is not freely released, and the moisture content is underestimated (figure 10).

The oven method is not a direct measure of the content of water. It measures weight loss that is assumed to be due to the loss of water. A basic analytical procedure is required to verify the temperatures and length of drying. The currently accepted procedure is the Karl Fisher procedure (figure 11) (Hart and Golumbic 1962). The moisture committee of the ISTA uses this procedure in its work to standardize and validate the oven procedures.

Another widely used method to measure seed moisture is the electronic moisture meter.

Although there are numerous brands of electronic moisture meters on the market, not all of them will work for tree and shrub seeds (figure 12), and those that do will not have calibrations for tree seeds. Therefore, conversion charts must be developed for them by testing samples with high to low moisture contents with both the meter and the oven. A linear regression between the oven and meter readings is calculated, and the conversion chart predicted from this regression (Bonner 1981; Hart and Golumbic 1966; Jones 1960; Karrfalt 1987; Lanquist 1965). These meters provide quick results, are nondestructive to the seed, and are usually accurate to within ± 1% of the moisture estimated by the oven method.

Purity, Noxious Weed Content, and Seed Weight Tests

Purity, noxious weed content, and seed weight tests are sometimes called physical tests because they do not relate to viability. These tests are described individually as follows.

Purity Analysis

After samples for the moisture-content test are withdrawn, the remainder of the submitted sample should be mixed and divided to obtain the working sample, which contains the minimum weight for conducting a purity analysis. Each species has its own specified minimum weight, which has been determined to contain 2,500 seeds. The mixing and dividing should be done in the same way as described in the sampling section for drawing the submitted sample from the composite sample. However, at this point it is necessary to be very close to the minimum weight for 2 reasons. First, the analyst does not want to examine more seeds than necessary, and second, the accuracy of the test is evaluated using tolerance tables that were developed using these minimum weights. Using substantially more seeds than the minimum will invalidate the use of these tables.

Purity is determined differently by each of the 2 major testing organizations. The ISTA rules specify a 3-part purity and the AOSA rules specify a 4-part purity. The ISTA purity values report percentages of pure seeds, other seeds, and inert materials. The AOSA purity values report percentages of pure seeds, weed seeds, other crop seeds, and inert materials. The pure-seed fraction consists of all those seeds that are of the kind specified on the seedlot=s label. Specific descriptions in the rules define Apure seeds,@ but basically the pure-seed fraction comprises whole seeds and seeds that are not more than half broken away. AOther seeds@ in the ISTA rule are all kinds of seeds other than those listed on the label. The AOSA rule makes a distinction between Acrop seeds@ and Aweed seeds@ and uses a detailed list (AOSA 1995) to specify when a species is a weed and when it is a crop. Weed seeds are mainly a problem in lots collected from nets or directly from the ground. Contaminated cleaning equipment can also result in weed seeds entering a seedlot. AInert matter@ is all other material that is not classified as a crop seed or other seed. It could include soil particles, stones, wire, small pieces of broken seeds, or other plant parts. Purity is calculated by dividing the weight of the of pure seeds by the total weight of all the fractions in the sample (figure 13) and is expressed as a percentage.

Purity work can often be tedious and very technical. Devices such as the mechanical purity board (figure 14) can speed up the procedure. The analyst must understand important

taxonomy principles and accurately use the seed herbarium (figure 15) to identify all the kinds of seeds in the sample.

Noxious Weed Examination

The noxious weed exam is a specialized purity examination. It is not a test traditionally associated with forest seeds but may become more common as the commercial exchange of native plants increases. A noxious weed is a highly aggressive competitor or a plant with other highly objectionable characteristics, such as being poisonous. It is so offensive it has been put on a noxious weed list compiled by an individual state or the federal government. A noxious weed exam is made solely to identify the number of noxious weed seeds found in the sample. Nothing else is noted in this exam. The presence of any noxious weed seeds makes it illegal to sell the seeds until the noxious weeds have been removed. The sample size for a noxious weed examination is 25,000 seeds.

Seed Weight Determination

The number of seeds per unit weight (kilogram and gram or pound and ounce) is determined on the pure-seed fraction from the purity test. This test is called the seed weight determination in the ISTA rules. It is made by counting out 8 replicates of 100 seeds and weighing them to the same precision as the weights for the purity test. The coefficient of variation for these 8 values is computed. This coefficient cannot be greater than 6 for chaffy seeds or greater than 4 for all other seeds. Otherwise, an additional 8 replications need to be counted and weighed and combined with the first 8 weights. All 16 weights are then used to compute the mean. Any weight diverging from the mean by more than 2 standard deviations is discarded; only the remaining weights are used to compute the number of seeds per unit weight.

Seeds can be counted by hand, with a counting tray, a shutter box, or a vacuum counter (figure 16). When seeds are counted by hand, it is usually best to count out the appropriate number of piles of 10, 20, or 50 seeds, in order not to lose one=s place. A counting tray is simply a block of wood or plastic with impressions drilled into it to hold the seeds as they are poured across the plate. The counting tray may or may not be faster than counting by hand, depending on the seeds= size and shape of, which determine how many double- or triple-seeded holes must be thinned by hand. Using the shutter tray is similar to using the counting tray, but the shutter tray is emptied by sliding the bottom tray rather than turning it over. For uniformly sized seeds, using either the counting tray or the shutter tray can speed up counting considerably.

The vacuum counter is probably the fastest and one of the most common ways to count seeds in the laboratory. The vacuum counter is made of an acrylic (or sometimes metal) plate that is drilled with 25, 50, or 100 holes and attached to a hollowed-out second plate. A vacuum line is attached to the head and a shut-off valve controls the application of the vacuum. To use the vacuum counter, the seeds are spread out loosely in a 1-seed-deep layer, the counter is placed on top of the seeds, and the vacuum is then turned on. Moving the counting head back and forth for about the diameter of a seed will bring the seeds into contact with a vacant hole. With the vacuum still on, the seeds can be transferred to a dish for weighing or to a germination container

(figure 17). Some users of vacuum counters report a tendency for lighter seeds, such as empty or partially filled seeds, to be picked up in preference to heavier seeds. To eliminate this problem, this device must be used according to the procedure described above.

Seed weights are sometimes determined with an electronic counter (figure 18). The ISTA rule calls for counting all pure seeds in the working sample when this is done. No error-check is then made. A recent internal report made by the Seed Count Committee of the Association of Official Seed Analysts, augmented by the author=s personal observations, suggests caution in the use of electronic counters for seed weight determinations. A high potential for error in counts exists. If carefully calibrated, these machines can count quite accurately, but the machines need to be adjusted and used correctly. A thorough evaluation of the degree of desired accuracy and the amount of time required to achieve it needs to be made before deciding to use the electronic counter.

Germination Testing

Germination testing is designed to estimate the maximum number of seeds that will produce a normal seedling and to give results that are as repeatable as possible. Without uniform procedures, there would be no standard on which to base the value of seedlots for commercial transactions and the seed trade would be chaotic and filled with dispute. Germination also tells a grower about a seedlot=s potential. A seedlot with 80% germination cannot produce more than 80 seedlings/100 seeds. Therefore, if 100 seedlings are needed, a minimum of 125 seeds must be planted (100/.80 = 125). How to use test data to compute sowing rates is presented in detail in chapter 7 (Nursery Practices) and later in this chapter in the section on the use of test data.

The germination test is conducted on the pure-seed fraction from the purity test. Both the AOSA and ISTA prescribe the use of 4 replications of 100 seeds. These replications can either be planted 1 to a container (figure 19), 2 to a container, or all on 1 tray. Alternatively, the 4 replications can be further divided into smaller replications, but the total number of seeds tested must remain 400 to remain in compliance with the rules. If fewer than 400 seeds are available, then the number of seeds per replication should be reduced so that an equal number of seeds is present in each of the 4 replications. Using fewer than 100 seeds in a replication is not according to the rules, and the test would thus be unofficial. However, it is better statistically to have 4 replications of 50 seeds each rather than 2 replications of 100 seeds each. The 4 replications are then placed under optimal germination conditions for the period specified in the rules. Germination is the number of normal seedlings produced from 100 pure seeds expressed as a percentage. A normal seedling has all the essential plant structures necessary for the plant to continue to grow normally under favorable conditions (AOSA 1996; ISTA 1996).

Seeds can be planted in a number of ways. They can be scattered or placed one at a time with forceps, although more generally a vacuum counter or other type of planting plate is used for speed and to ensure even spacing of the seeds. The vacuum counter is the most expedient technique, because it can handle a variety of seed sizes (figure 17). Counting devices are described in the seed weight discussion above. Seed should be hand-planted only when counting devices cannot be used in order to save time.

Seeds can be germinated on various media. Sand, sand and perlite mixtures, potting

mixtures, soil, and various papersCblue blotters, white blotters, or crepe-cellulose papersCcan be used (figure 20). Testing rules, however, specify what is an acceptable medium for the kinds of seeds tested. Specifying the medium helps assure uniformity in test results. The blotters resist penetration by the roots of the plants, whereas the crepe-cellulose paper allows for root penetration. Blotters offer the advantage of keeping the roots where the analyst can actually see them for evaluation, but if a seedling is very large it will fall over and tangle with other seedlings, making counts difficult. The media also differ in their water-holding ability. Blotters usually need to be watered several times during the test, whereas crepe-cellulose paper, sand, sand mixtures, potting soils, and soil are absorbent enough to hold all the water the seeds need for up to 3 months, if kept in a moisture-proof container. Watering the medium can be done by hand or by machine. Watering by hand is usually done using a squeeze bottle or a small hose from the tap and requires subjectivity on the analyst=s part to estimate that the correct amount of water has been applied. Too much or too little is harmful, but in most cases there is wide latitude in the amount that will give optimal results (Belcher 1975). Machines for watering include automatic pipetting machines (figure 21) or small traveling spray booms. Both save a great amount of time if many tests are conducted and, once adjusted, take all the guesswork out of applying the correct amount. These machines should be checked periodically, however, to verify that they are in fact applying the desired amount of water.

Germination tests should be run in cabinets or rooms that meet exacting requirements for temperature and light control in order to make accurate and repeatable estimates. Temperatures should be carefully checked throughout the chamber at the level of the substrate to be sure there are no places that deviate from the desired temperature by more than 1 °C. Poor air circulation and hot spots from lights or light ballasts are the most common causes of temperatures that are too high or too low. The temperature at which the germination chamber is set depends on the species being tested. Many species do well at an alternating 20 and 30 °C. For this regime, the chamber is held for 16 hours at 20 °C and for the remaining 8 hours of the day at 30 °C. Other possibilities are constant temperatures of 15, 20, or 22 °C, with light usually supplied for either 8 or 16 hours. When temperatures alternate, the light is provided during the higher temperature to follow a natural cycle of light and temperature. Sources of light need to contain abundant amounts of blue and/or red light but not far red light because far red light is known to inhibit germination. Cool white flourescent lamps are most commonly used. The temperature/light regime used for a germination test is determined by experiments that germinate the same seedlot at different temperature/light combinations. The combination that supports the highest percentage of germination in the most reasonable time is the one that is then adopted in the rules for testing.

Dormancy is the condition of a seed that prevents it from germinating when it is placed in conditions that are favorable for germination. (For a discussion of dormancy, see chapter 1.) Dormancy must be overcome in order to conduct the germination test, just as when trying to grow seedlings. Prechilling (also called prechill and traditionally called stratification) is the procedure most used for breaking dormancy in forest seeds. The seeds are held in moist conditions at temperatures between 0 and 3 °C. Prechilling can be accomplished in 1 of 3 basic ways. In the first, the seeds can be planted on moistened germination medium in sealed containers and then put in the cold. In the second, the seeds can be placed in a moist medium, placed in the cold, and then at the end of the prechilling period, planted on the germination

medium. In the third method (similar to the second), the seeds are soaked for 16 to 48 hours in water to become fully imbibed, placed in a moisture-proof container, held in the cold for the specified period, and then planted on the germination medium. This last procedure is sometimes called naked stratification, because no moisture-holding medium is used (figure 22). How long seeds are held in prechill varies widely by species and genetic source of the seedlot. The period can range from 10 days to many months. For some species, a warm period preceding the cold period is required. This is called warm stratification or warm incubation. Western white pine *(Pinus monticola* Dougl. ex D. Don) (Anderson and Wilson 1966) and European ash (*Fraxinus excelsior* L.) (Piotto 1994) have been reported as requiring this warm-cold stratification .

A species that does not require prechilling is called nondormant. If 10 to 14 days of prechilling are needed, the dormancy would be considered light. If 30 to 60 days of prechilling are required to break the dormancy, it would be considered moderate. More than 60 days of prechilling classifies the seedlot as highly or strongly dormant. The degree of dormancy varies within the seedlot of even lightly dormant species; some seeds germinate without prechilling, whereas other seeds in the same lot will not germinate until they are prechilled. However, the term variable dormancy is usually reserved for seedlots in which some seeds germinate during prechilling, whereas other seeds in the same lot will not germinate even after being placed in favorable germination conditions. Species that fit the deep and variable dormancy category are Rocky Mountain juniper (*Juniperius scopulorum* Sarg.) and basswood (*Tilia americana* L.).

Because of the above-mentioned variation in dormancy, seedlots will often be tested with and without prechilling or with varying lengths of prechilling. Such tests are referred to as paired or double tests; usually only 2 tests are done. More tests, of course, can be and are done with some seedlots. This type of testing can determine the presence of dormancy, the strength of dormancy, or a weakness in the seeds (1995). When the seedlot has the same germination with and without prechilling, it is said to be nondormant. When the germination is increased with prechilling, the seedlot is classified as dormant; the longer the prechilling period needed, the stronger the dormancy is said to be. A decrease in germination with prechilling is an indication of weakness in the seeds. This last condition is similar to the situation of the type of vigor test known as the cold test, which is described in the following section.

Prechilling is not the only treatment to break dormancy. Light is useful to break dormancy and can reduce the need for prechilling. Birches (*Betula* L.) and loblolly pine (*Pinus taeda* L.) are prime examples where light helps break dormancy. Seedcoat dormancy is treated by scarifying the seedcoat with either acid, bleach or mechanical means. Chemical stimulates such as gibberellins or potassium nitrate have been little used with forest tree seeds.

Vigor Testing

Sometimes standardized laboratory germination procedures are criticized as not predicting field performance very well (Moreno 1985; Stein 1967). These critics suggest using a variety of test conditions to find an optimum for each seedlot. The problem in predicting field germination is that it is impossible to predict the weather with the necessary precision. Vigor testing is one possible solution. The vigor test does not predict performance for a particular set of fluctuations; rather, it predicts the general ability of a seedlot to germinate normally over a range

of adverse conditions. Its purpose is to differentiate seedlots, with essentially equal germination, according to their ability to germinate well in spite of adversity. Figure 23 illustrates the relationship between vigor and germination. As seeds age and begin to weaken and die, vigor declines before germination test results decline (Belcher 1978; Justice and Bass 1978).

Like germination tests, vigor tests are conducted under standardized conditions in order for the results to be repeatable and useful in the field. A vigor test cannot make up for poor practices that unnecessarily increase environmental variation in the field; such poor practices can be major sources of disparity between laboratory and field germination. Uniform sowing depth and watering, as well as sowing only on soil at the minimum acceptable soil temperature Call help make field germination more predictable.

The most common vigor tests in agriculture are the cold test, the accelerated aging test, the conductivity test, and the tetrazolium test. These 4 tests have not been used very much for forestry. In addition to these tests, speed of germination as expressed in a number of formulas has been put forward for use in forestry as a vigor test. Despite the potential benefit for tree seed nurseries, the science and technology are not advanced enough to permit the practical application of vigor testing with forest species.

The cold test is done by planting the seed in damp soil and then holding the germination tray at 10 °C for 7 days. This test mimics the cool damp conditions of soil in early spring. At the end of the period, the germination trays are transferred to the appropriate temperature for germination. The higher the percentage of germination, the more vigorous the seed is said to be. High-germinating lots have the needed strength or vigor to pass the period of stress and still have energy for high germination when conditions are favorable. This is analogous to the case of the tree seedlot that drops in germination following prechilling. Those that drop in germination after prechilling are weak.

The accelerated aging test is conducted with the stress of high temperature and moisture. The given weight of seeds is placed in a small box with a screen tray that suspends the seeds over a reservoir of water (figure 24). These boxes are then placed in an aging chamber at 40 to 43 °C for 72 to 288 hours, depending on the species. Whichever temperature is chosen in this range, the variation must be virtually nil to ensure repeatability of the results. The water-jacketed incubator has been determined by organized tests among laboratories to be the best device to give this necessary strong control over the test conditions. At the end of the period the seeds are planted and tested for germination under the standard conditions.

The electrical conductivity test has been widely tested in agriculture but has not been adopted as routine practice except in a few specialized areas. In this procedure, seeds are soaked individually or in bulk. Deteriorated or dead seeds leak electrolytes more readily than high-vigor seeds. This greater leakage causes the water to have a higher conductivity, which can be measured with a conductivity meter (figure 25). Bonner and Agmata-Paliwal (1992) reported on the use of conductivity for tree seeds and found that results have poor repeatability for precise estimates but possibly would work for general estimates of classes as poor, low, intermediate, or high viability.

Several statistics have been put forward to use speed of germination as an indicator of vigor. The faster a seedlot completes germination or reaches its peak the more vigorous it is said to be. The simplest indicator is days to 90% of total. For example, if the final germination is

88%, the indicator would be how many days it takes to reach 79% germination. A lot that reaches 79% in 12 days would be more vigorous than one that takes 16 days. To use this statistic, counts must be made quite frequently, even daily, or the data must be interpolated to determine the number of days to the specified germination.

Czabator=s factor (1962), developed for use with southern pines, combines the maximum daily average germination, called the peak value, and the average daily germination at the end of the test to form one statistic called the germination value. Germination is counted frequently, at least every third day, and the cumulative germination on each day is divided by the number of days that the test has been run in order to compute the mean daily germination for that day. For example, if on day 22 the cumulative germination is 88, the mean daily germination is 4. This mean daily germination increases with each day of germination until the period of maximum germination has ended and then decreases. The largest value of the mean daily germination is called the peak value. Figure 26 shows a graph of a typical germination. Initially only a few seeds germinate, followed by a period of rapid progress, and finally a slow-down period and an end of germination altogether. Germination value is computed by multiplying the peak value by the mean daily germination. Lots that have higher germination values are generally considered more vigorous.

Another characteristic of more vigorous lots is that they store for longer periods of time without loss of germination. Therefore, if 2 seedlots have equal germinations, the one with the lower vigor might be considered for first use, because the germination of this lot will likely decrease faster than a lot of higher vigor. This approach would give the greatest potential number of seedlings. Lower vigor seedlots will loose viability even under ideal conditions in the freezer.

Tetrazolium staining has been tried also as a vigor test (Moore 1976). Because of the highly subjective nature of this test and the great amount of experience it requires to administer, it has never been widely used as a vigor test and never successfully with forest plants. As stated in the next section, tetrazolium can be used to successfully estimate viability for very dormant species or for other hard-to-germinate species.

The problem with vigor analysis is that it has proven to be difficult to standardize. This difficulty is apparently because the test conditions are so exacting. In tests involving germination, the temperature must be very tightly controlled. Just a degree or two difference in temperature can change the speed of germination, effecting the value of those statistics that rely on germination speed. The accelerated aging test was difficult to standardize until chambers were developed that had virtually no variation in temperature. Difficulty in standardizing laboratory tests and the lack of clear and consistent interpretation to the field has prevented the operational use of vigor testing.

For a more complete list of literature references and a detailed explanation of the vigor testing procedures, refer to the Association of Official Seed Analysts= Seed Vigor Testing Handbook (AOSA 1983) and the Handbook of Vigour Test Methods prepared by the International Seed Testing Association (ISTA 1995). Bonner (1998) has also made a thorough review of vigor testing specifically for tree seeds.

Chemical Staining for Viability

The tetrazolium staining procedure mentioned in the vigor section is useful in estimating the viability of dormant seeds, especially very dormant seed. This test involves soaking the seed first in water to fully imbibe the seed and soften it for cutting. A moistened seed will take up the stain more rapidly. A variety of methods are used to open seeds. It is extremely important that no damage occur to the embryonic axis when a seed is cut. The embryonic axis is the radical and the plumule. The meristematic regions are here, and their condition needs to remain unaltered until they are carefully examined. These are the areas where the embryo must grow in order to produce a normal seedling. Usually forceps and sharp single-edged razor blades are used to cut open the seeds (figure 27). For seeds with harder or stony seedcoats, a variety of vises, hammers, and clippers are used to cut through or remove the seedcoat (figure 28).

The solution that is used to make a tetrazolium (TZ) test is colorless. It is made by dissolving 2,3,5-triphenol tetrazolium chloride in a phosphate buffer at pH 7.4, which is the optimum pH for the TZ reaction. The buffer is necessary to compensate for any pH imbalance in the TZ salt, the water, or possibly the seeds. The colorless solution is taken up by the prepared seeds and then reacts with respiratory enzymes (that is, dehydrogenases) to form an insoluble light pink (magenta) precipitant. Tissues that are alive and respiring will stain, and those that are not alive will not. For a detailed discussion of this procedure, refer to the AOSA Handbook on Tetrazolium Testing (AOSA 2000). The TZ test can be completed in 4 to 48 hours, depending on the amount of preparation time required and the rate of staining.

Tetrazolium staining has proven useful with many species that have deep dormancy, including tuliptree (*Liriodendron tulipifera* L.), baldcypress (*Taxodium distichum* (L.) Rich.), Rocky Mountain juniper, and sumac (*Rhus* spp. L.) species. However, for a few species with very deep dormancy, there will be no staining (Vivrette 1995) unless the seeds are prechilled.

Excised Embryo Testing

The excised embryo test is done on the embryo after it is removed from the seed (Flemion 1948; Heit 1955). In this germination test, the embryo has been freed from the restriction of the seedcoat and nutritive tissue (figure 29). Therefore, a germination that would take many months and be incomplete can be complete in 10 to 14 days. The ashes (*Fraxinus* L.), maples (*Acer* L.), and cherries and plums (*Prunus* L.) are some of the genera that are tested by embryo excision. Because the embryos are very vulnerable to infection once excised, the test must be done under strictly clean (axenic) conditions. The work surface and all tools, hands, and germination dishes should be washed carefully, perhaps with absolute ethanol. If good embryos easily mold, then cleaning procedures must be reviewed for effectiveness and the work area examined for sources of microbial contamination. Generally, however, sterilization procedures such as autoclaving are not required. The procedures for excising the embryos are similar to those used in preparing seeds for tetrazolium. Greater care is needed, however, because the embryo must be removed intact without any significant injury or broken apart. The main advantage of this method over the TZ test is that the evaluation is less subjective; the growing embryo is actually observed in most cases. Therefore, a direct reading on the growth potential of the seedlot can be obtained.

A complete excision is not always required. Russian-olive (*Elaeagnus angustifolia* L.) (Belcher and Karrfalt 1979) and western white pine (Hoff and Steinhoff 1986) respond well to

only partial removal of the seedcoat. The white pine requires prechilling before the seedcoat is cut open.

X-radiography

X-radiography is very useful in forest seed analysis. It provides a very rapid and accurate analysis of the internal structure of seeds, identifying empty, insect-damaged, or poorly developed seeds (figure 30). It is an immense help in judging maturity, determining how many bad seeds should be remove, and detecting any mechanical injury. It is more accurate than cutting tests in many cases, requires much less time, and is nondestructive (AOSA 1979; Simak and others 1989). X-radiography was first applied to tree seeds by Simak in Sweden. The use of contrast agents has improved the ability of the x-ray test to discriminate between viable and nonviable seeds with some species (Kamra 1963; Simak 1957; Vozzo 1978). A contrast agent enters damaged areas of the seed differentially from nondamaged areas, making the damaged areas more radiopaque. They will then appear as bright areas on the radiograph. Aqueous solutions of heavy salts such as iodine or barium chloride and vaporous agents such as chloroform have been used as contrast agents.

Radiographs can be made on Polaroid7 film, x-ray paper, or x-ray film. Polaroid film is useful if no darkroom is available,because the film is developed in the light, just like a Polaroid photograph. The disadvantage of Polaroid is the high cost, short shelf life, and lack of detail. X-ray paper is fast to use but does require a simple darkroom. It is less expensive than Polaroid, has a shelf life of several years in cold storage (3 °C), and much better resolution. The best resolution is obtained with x-ray film. X-ray film, however, is more expensive and slower to develop (over 45 minutes) and a light table is required to see the images. Usually the film or paper is placed in a paper or vinyl cassette so that it may be handled in the light. This cassette can result in some loss of clarity of the image, especially with small seeds; using the film in the dark where the seeds can be laid directly on the emulsion gives a noticeably superior image.

Seed work is usually done with x-rays in the range of 10 to 30 kvp (that is, kilovolt potential), which is is the amount of penetrating power the x-rays have. The exact kilovolt potential depends on the equipment and the seeds in question. Trial and error is necessary to find the best combination. Too high a kilovolt potential and the seeds will not be visible or will appear too dark. Too low a kilovolt potential, and the image will lack detail and be too light. Some small seeds need to be x-rayed at a low kilovolt potential to give the correct penetration but need a long exposure to produce a radiograph with enough density to provide good contrast. X-ray inspection cabinets are manufactured that operate in this very low kilovolt potential range for examining small items such as seeds. They are designed for total protection of the operators, with complete lead shielding and safety interlocks on the door (figure 31).

X-radiography has proven useful for studying the seeds from many wild species, which often can be empty or poorly formed. Some laboratories test every lot of seeds they receive with x-rays and get a good initial evaluation. X-radiography can be of great value in evaluating germination test results, because it is much faster than cutting open seeds that failed to germinate. Empty seeds will never germinate, and damaged or poorly developed seeds will seldom

germinate. The excised embryo or tetrazolium test for difficult-to-cut seeds can be speeded up by x-radiography. The seeds are first placed on the x-ray film or paper in a manner that will allow the comparison of the exact image to the exact seed. This is done by placing the seeds on an additional piece of paper before placing the paper on the x-ray film or paper. If orientation of the seed is important, as in double-seeded fruits such as dogwood, the seeds can be placed on adhesive tape and that then laid on the paper. The seeds should be oriented so that both seeds in the fruit can be viewed and the tape prevents them from turning. After the radiograph is made, the seeds are gently slipped off the x-ray paper so that the seeds are kept in order for cutting. Only those seeds that are morphologically sound in the radiograph need to be cut.

Other Quick Tests

As stated in the introduction, cutting tests are very limited in their application. However, they can provide useful information on full seed percentages and the condition of the internal structures. For example, color of the tissue cannot be determined in a radiograph, which is only black and white. Seeds that are cut and found to be dark are not likely to germinate. New and unfamiliar images in a radiograph require cutting the seed to determine what is actually in the seed. With slash and longleaf pines, cutting can reveal embryos that have initiated chlorophyll (turned green), a result of a seedlot having been held too long at high moisture. This is a sure sign of a weakened seedlot.

Hydrogen peroxide (H_2O_2) has been used as a quick test for western conifers (Ching and Parker 1958). In this test, the seeds are floated in solution of 1.0% hydrogen peroxide overnight. The radicle ends are then clipped and the seeds incubated in the dark at 20 to 30 °C for 10 to 12 days. Counts of germinates are made at 3 to 4 days and at 10 to 12 days. The hydrogen peroxide solution is changed at the first count.

Sowing Rates

A sowing rate is the amount of seeds sown in a unit area of nursery bed to produce the desired number of seedlings. The following formulas show how seed test data are used to determine this rate.

Weight of seeds to sow in a nursery bed (width x length) is equal to

$$\frac{\text{bed width x bed length x seedlings desired per area}}{\text{germination x seeds per weight x purity x survival factor}}$$

Number of seeds needed to sow per area of nursery bed is equal to

$$\text{seedlings desired per area}$$

germination x purity x survival factor

In both of these formulas, the survival factor is the ratio of the number of seedlings expected to the number of viable seeds planted. It is derived from experience in the given nursery and should be constantly updated with new information collected from history plots. History plots are permanent sample plots in a nursery bed used for carefully monitoring the number of seeds sown and the number and quality of seedlings produced (Landis and Karrfalt 1987). For example, if 100 seeds are sown on a square foot, germination is 80% in the laboratory, and 60 seedlings actually grow on the square foot, then the survival factor is 60/80 or .75 (75%).

Computing sowing rates for containers is somewhat different, because we must predict the probability of an empty cell in the container. The probability that a container cell is empty is equal to 1 minus the probability that at least 1 seedling is in the cell. Sowing 1 seed/cell, this probability is 1 minus germination. With a 90% germination, the probability of an empty cell following single- seed sowing is 0.1. In sowing 2 seeds/cell, the probability of no seedling in a cell drops to .01, but now there are 81 cells (.90 x .90) out of 100 that will have 2 seedlings/cell. Double seedlings per cell requires thinning to 1 seedling/cell for proper growth. Thus, in container nurseries it is necessary to choose between empty spaces and thinning. In this example, to go from 10 empty cells to 1 empty cell/100 cells, 81 seeds were wasted. For 10,000 seedlings, 8,100 seeds would be wasted which would be 112 to 224 g (3.9 to 7.8 oz.) of seeds when the seeds per kilo is 72,300 (32,900 seeds/lb.). Thinning also requires more labor and may be dysgenic by favoring early germinating genotypes. Sowing extra containers is another strategy followed to compensate for empty cells. The empty cells are still present but enough seedlings are produced without the problems of thinning.

The purity and seed per weight are still important to the container grower, because they will be used to compute the amount of seed to prepare. This formula tells how many seeds are in a unit weight of seed.

weight of seeds x purity x no. of seeds per unit weight

Example: 1 kg of seeds at 98% purity, 33,000 seeds /kg
1 kg x 0.98 x 33,000 pure seeds/kg = 32,300 pure seeds

or 1 lb of seed at 98% purity, with 15,000 pure seeds/lb
1 lb x 0.98 x 15,000 seeds/lb = 14,700 pure seeds

To sow 10,000 cells with 1 seed each, 10,000 seeds are needed, which is 10,000 seeds divided by 32,300 seeds/kg = 0.31 kg (10,000 divided by 14,700 = .69 lb). Double sowing 10,000 cells would take 2 x 0.31 = 0.62 kg (2 x .69 = 1.4 lb). Combining these steps into one formula gives the result below:

$$\frac{\text{number of seeds to sow per cell x number of cells to sow}}{}$$

purity x seeds per unit weight

In the double-sowing example, this would be 2 x 10,000 divided by .98 x 33,000 seeds/kg (.98 x 14,700 seeds/lb) for a total of 0.62 kg (1.4 lb) of seed required.

Buying and Selling Seeds

Current test data are essential. To be current, the data should not be more than 9 months old. The more recent the test, the more likely it is to reflect the true condition of the seed when the buyer takes possession of it. Ideally the tests should be run by a disinterested third-party laboratory that is well qualified to do the tests. Never accept the results of informal analysis, such as the cut test, as a true measure of the worth of the seedlot.

Which tests are important to request, and how should they be used? Moisture content is important for 2 reasons. First, the seeds need to be at a proper storage moisture content to ensure viability. Orthodox seeds need to have a moisture content below 10% and recalcitrants usually above 25%. Second, it must be remembered that extra water can be added to the seeds and distort the true value of the lot. One kilogram of a seedlot with 10,000 seeds/kg (22,000 seeds/lb) at 7% moisture content would contain 70 g (2.5 oz) of water and 930 g (32.6 oz) of dry seeds. A similar lot of 10,000 seeds/kg at 9% moisture would have 90 g (3.2 oz) of water and 910 g (31.9 oz) of dry seeds. The second lot actually contains 2% fewer seeds. Because the water is free, adding extra moisture can be a good way for the seller to increase profits. And although both seedlots might appear to have seeds of the same size, the lot with higher moisture would actually have slightly smaller seeds.

The number of pure live seeds per weight is a calculation that is often helpful in assessing the value of a seedlot. In this procedure, germination, purity, and seed weight are all considered. Consider a seedlot with germination of 90%, purity of 98%, seed weight of 18,600 seeds/kg (8,500 seeds/lb). The number of pure live seeds per kilogram is then .90 x .98 x 18,600 = 16,400 seeds/kg (7,500 seeds/lb). This is the same value as a lot that has 95% germination, 96% purity, and 18,000 seeds/kg (8,200 seeds/lb), (.95 x .96 x 18,000 = 16,400) but a higher value than a lot with 97% germination, 84% purity, and 17,400 seeds/kg (7,900 seeds/lb), (.97 x .84 x 17,400 = 14,200). Number of pure live seeds per weight tells the grower the potential number of seedlings and removes at least some ambiguity in comparing the value of different seedlots. If the maximum number of potential plants is the most important factor, the first 2 lots are superior to the third, although the third has a higher viability. Alternatively, as might be the case for a container nursery, the high germination could be the most important factor, and the third lot would be chosen over the first two, even though potentially fewer trees can be produced from it.

Minimum standards are usually set for all quality values. Those minimums depend on the type of nursery, the generally available quality for a species, the desirability of the seed source, and other factors. In general, the higher its quality, the more a seedlot is worth in the nursery. High germination is of great value in a bareroot nursery but indispensable to the container grower, who seeks to avoid wasting seeds by double-sowing or having empty growing space in single sowing. However, for some species, 60% germination might well be typical and expecting 98% germination is not reasonable.

Test Limitations and Variation

Standardized laboratory tests are designed to give maximum values with minimum variation, allowing the results to be repeated. Without repeatability, there would be no standard by which to compare different seedlots. Assessments would become even more difficult if seed tests were conducted in random fashion to mimic field conditions. Although the correlation between laboratory and field germinations is frequently low, experience has shown that seedlots with higher germination scores will, over time, give more germination in the field. Further improvement in predicting field performance may result from improvements in the techniques of vigor testing.

Because seeds are biological, they usually are quite variable in size and performance. Natural things simply show more variation than is usually seen in manufactured items. Test results, therefore, can vary more than perhaps is expected and still be accurate. For example, a seed weight of 16,000 seeds/kg (7,300 seeds/lb) might be reported as 15,600 (7,100) in a second test, and both results are in fact accurate. Tolerance tables can guide decisions on whether test results are comparable. Some of these tables are suitable for all types of seeds, whereas others vary depending on the type of test and the kind of seeds tested.

Scheduling Seed Tests

When should a seed test be conducted? Tests are needed to formally determine the quality of the seedlot upon completion of the conditioning process. All variables then need to be measured: germination, seed weight, purity, and moisture content. Moisture needs to be monitored during storage to be sure it is being properly maintained. If there are no changes in moisture content, then seed weight and purity will not change and viability will change very slowly, if at all. Some annual monitoring of seed moisture is necessary to ensure that storage conditions are being adequately maintained. The viability can be retested at 3- to 5-year intervals with a current test always done no more than 6 to 9 months before sowing. If a longer time passes before sowing, some deterioration could occur, resulting in changes in germination. Determining viability in some seeds takes a long time, and thus it is important to schedule adequate lead time into the production schedule.

Commercial Trade of Tree, Shrub, and Native Plant Seeds

Official rules for testing seeds developed by the International Seed Testing Association or the Association of Official Seed Analysts are very important to the orderly buying and selling of seeds. These rules give standardized procedures that can be repeated with acceptable variation no matter what laboratory conducts the test. In addition, the seed testing associations conduct comparative tests among themselves to verify that the procedures are being applied uniformly and within tolerable limits of error. Such a system is important: it assures sellers that they are offering good seed for sale, and it reassures buyers by giving them reliable information on which to base their purchases. There are also consumer protection seed laws in some countries or states

requiring that seeds offered for sale meet certain minimum standards or be accurately labeled as to their quality. Without a repeatable system of testing procedures, such laws would be impossible to enforce. Both the consumer and the reputable seed dealer would suffer. Uniformity of the rules combined with uniformity in applying the rules equals order in the marketplace.

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Figure 1—Seed probes are used to sample free-flowing seeds.

Figure 2—Seeds caught in the gates of the seed probe must not be cut when the gates are closed.

Figure 3—The seed probe is emptied by pouring the seeds out the top.

Figure 4—An open hand is inserted into a seedlot to take a sample for testing.

Figure 5—Seed can be hand-mixed before withdrawing a submitted sample from a composite sample.

Figure 6—The composite sample is divided systematically into quarters, eighths, sixteenths, and smaller fractions to obtain the submitted sample at the seed storage plant or the working sample in the laboratory.

Figure 7—A soil divider (L) and a gamet divider (R): two devices to systematically mix and divide seed samples.

Figure 8—Two seed samples are tested to measure the moisture content of a seedlot.

Figure 9—A convection oven, desiccator, and analytical balance are used to conduct a seed moisture test.

Figure 10—Cutting large seeds open before drying them in the moisture test is necessary to release all the moisture.

Figure 11—The Karl Fisher apparatus is used as the analytical standard for determining seed moisture content.

Figure 12—Electronic moisture testers can give a quick and reasonably accurate estimate of seed moisture.

Figure 13—A purity sample is divided into its component parts.

Figure 14—A mechanical purity board can reduce the time required to conduct a purity analysis.

Figure 15—Seed herbaria are used to make positive identification of the species of seed tested.

Figure 16—Seeds can be counted sometimes more quickly using a counting tray, a shutter box, or a vacuum counter.

Figure 17—A vacuum counter is often used to count out seeds for weight determinations and for planting germination tests.

Figure 18—An electronic seed counter is sometimes used to estimate the number of seeds per weight (measured in either kilograms or pounds.

Figure 19—Five germination tests, each composed of four dishes containing 100 seeds. The dishes are stacked for transport and prechilling.

Figure 20—Seeds are germinated on various media, from left to right: crepe-cellulose paper (Kimpak7), blue blotters, sand–perlite mixtures, and potting soil.

Figure 21—An automatic pipetting machine can help to uniformly and rapidly water germination dishes.

Figure 22—Seeds can be prechilled on a germination medium, in separate medium, or in a plastic bag.

Figure 23—As seed viability decreases, the proportion of live low-vigor seeds increases.

Figure 24—The accelerated aging test is conducted by placing seeds in a plastic box with a water reservoir and holding them at 40 °C for 72 hours.

Figure 25—The conductivity meter is used to measure seed viability or vigor by estimating the amount of cations lost from deteriorating seeds.

Figure 26—Germination curves of 3 pairs of seedlots: high germination, moderate germination, and low germination. The upper curve in each pair represents the more vigorous lot in the pair because the germination is completed sooner.

Figure 27—Seeds are cut open carefully to prepare them for tetrazolium staining.

Figure 28—Vises, hammers, or clippers are used to cut through or remove hard or stony seedcoats for conducting tetrazolium or excised embryo tests.

Figure 29—Peach (*Prunus persica*): embryos have been removed from their seedcoats for an excised embryo test of viability.

Figure 30—An x-radiograph can be used to quickly determine how many seeds are empty, damaged, or poorly developed.

Figure 31—A cabinet x-ray system is a safe and simple way to make radiographs of seeds.